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Fingerprint and high-wavenumber Raman spectroscopy in a human-swine coronary xenograft in vivo

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Abstract. Intracoronary Raman spectroscopy could open new avenues for the study and management of coronary artery disease due to its potential to measure the chemical and molecular composition of coronary atherosclerotic lesions. We have fabricated and tested a 1.5-mm-diameter (4.5 Fr) Raman catheter capable of collecting Raman spectra in both the fingerprint (400–1800 cm⁻¹) and high-wavenumber (2400–3800 cm⁻¹) regions. Spectra were acquired in vivo, using a human-swine xenograft model, in which diseased human coronary arteries are grafted onto a living swine heart, replicating the disease and dynamic environment of the human circulatory system, including pulsatile flow and motion. Results show that distinct spectral differences, corresponding to the morphology and chemical composition of the artery wall, can be identified by intracoronary Raman spectroscopy in vivo.© 2008 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2960015]

Keywords: Raman spectroscopy; intravascular catheter; atherosclerosis; coronary artery; xenograft; fiber optic probe.

We have constructed a prototype Raman catheter capable of acquiring both FP and HW spectra. The catheter was comprised of an inner optical core surrounded by a transparent outer-diameter stainless-steel tube was cut to provide a circular shortpass filter. The filter was bonded 830-nm cutoff m-diameter dielectric interference filter on a fused silica annular longpass filter has been recently investigated for arterial diagnosis ex vivo. The high-loss for two separate channels with distinct optical filters at the catheter’s distal tip presents a significant challenge for fabricating small-diameter, flexible Raman catheters suitable for intracoronary use. Several FP Raman probes have been demonstrated for atherosclerosis, all featuring a ring of collection fibers surrounding a central excitation fiber. The need for two separate channels with distinct optical filters and allowing HW Raman spectra to be acquired through a single fiber. While FP and HW Raman signals provide complementary chemical information, the diagnostic potentials of both regions have not been fully explored. The development of a single catheter capable of collecting both FP and HW Raman spectra will facilitate the investigation of the chemical and molecular information content that can be obtained from each wavenumber region alone and in combination.

We have constructed a prototype Raman catheter capable of acquiring both FP and HW spectra. The catheter was comprised of an inner optical core surrounded by a transparent nylon sheath [Fig. 1]. The optical core contained a central excitation fiber surrounded by an outer ring of six collection fibers. Each fiber was made of low OH, fused silica, with a 100-μm core diameter (Polymicro Technologies; Phoenix, AZ). The catheter utilized a custom-manufactured, monolithic 775-μm-diameter dielectric interference filter on a fused silica substrate (BARR Associates; Westford, MA), featuring a circular shortpass filter (830-nm cutoff) surrounded by an annular longpass filter (860-nm cutoff). The filter was bonded with epoxy to the distal end of the fiber bundle. A 1.5-mm (4.5 F) outer-diameter stainless-steel tube was cut to provide a groove, which served as an optical window [Fig. 1(c)]. The fiber bundle/filter unit was inserted into the tube and bonded, along with a 45° aluminum-coated rod mirror oriented to pro-
the formation of blood clots. After the graft was in place, Raman spectra were acquired from the grafted human artery in vivo, using illumination powers of 100 mW at 830 nm and 85 mW at 740 nm. FP and HW spectra were acquired over 40 frames at 0.25 s each for a total acquisition time of 10 s at each site, which enabled us to evaluate spectral quality as a function of integration time. Based on previously reported results,7,8 and ex vivo experiments performed in our laboratory,14 we believe that these laser fluences do not cause arterial tissue damage or blood coagulation. After each Raman measurement, the interrogated site (identified by visualizing laser light transmitted through the artery wall) was marked with green ink for subsequent correlation with histology.

Raman spectra were extracted from the collected raw spectra using established postprocessing methods,8 including frame averaging, system spectral response correction, and catheter and fluorescence background removal. First, a specified number of raw data frames were averaged together and divided by a white light reference spectrum. The tissue fluorescence spectrum was removed by subtracting a fifth (FP) or sixth (HW) order polynomial, and the catheter background was removed by subtracting a processed and scaled background spectrum that was acquired while holding the catheter in air. To determine the minimum necessary acquisition time, we averaged various numbers of frames together and analyzed the resulting processed Raman spectra. In Fig. 2, we show Raman spectra that correspond to an integration time of 4 s (16 frames).

Raman spectra from the two sites differ significantly. In the first site [Figs. 2(a) and 2(b)], the FP Raman spectrum contains a prominent peak at 960 cm⁻¹ (corresponding to the phosphate stretch of calcium hydroxyapatite) and minimal lipid. The Raman spectrum is consistent with histology, which demonstrates a large calcific nodule [Fig. 2(e)]. Spectra obtained at the second site [Figs. 2(d) and 2(e)] show peaks representative of adventitial triglycerides (CH₂ bends at 1301 and 1440 cm⁻¹ and C=C stretch at 1654 cm⁻¹ in the FP spectrum and ~2836 cm⁻¹ in the HW spectrum). There is an increased contribution from cholesterol (~2870 cm⁻¹) in the HW spectrum, which may correspond to a small amount of lipid staining seen on the Oil-red-O histology section [Fig. 2(f)]. A minute calcium hydroxyapatite signal is also present, which may result from the large, adjacent calcific nodule.

In the two samples shown, the arterial lumen is only marginally larger than the catheter diameter, and thus the catheter was nearly in contact with the lumen surface. We have performed ex vivo experiments suggesting that Raman spectra can be reliably obtained through a small distance of blood (<0.5 mm) and through a moderate distance of saline (<2 mm). Thus, in a larger-diameter artery, our Raman catheter may require a saline purge to clear blood and/or an additional mechanism to ensure contact with the arterial wall (e.g., a balloon).

In this letter, we have demonstrated a Raman catheter capable of acquiring both FP and HW Raman spectra in vivo. Compared to previously demonstrated fiber-based Raman probes, the design and fabrication of our catheter was simplified by the use of the single-piece, patterned dielectric filter module. While our current catheter has a relatively large outer diameter of 1.5 mm, the limiting factor is the diameter of the fiber bundle and filter (775 μm). Thus, the overall catheter...
diameter can be reduced to less than 1 mm by simply replacing the side-viewing mirror and outer sheath, which will bring the catheter diameter on par with that of commercially available intracoronary imaging catheters. Future catheter development efforts will focus on reducing the catheter’s diameter, scanning the inner optics with respect to the outer sheath, and increasing the Raman signal collection efficiency by improving the fiber bundle’s filling density and optimizing the optics at the distal end of the catheter.

Acknowledgments
This study was supported by Prescient Medical, Inc. A.H.C. was supported by a Ruth L. Kirschstein individual fellowship (Grant no. F31EB007169) from the National Institute of Biomedical Imaging and Bioengineering.

References